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Tick-borne rickettsiae in Guinea and Liberia

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ABSTRACT

While the high seroprevalence for the rickettsiae that cause spotted fevers and the multiple pathogenic rickettsiae is known, the data on the distribution of rickettsial diseases in Africa are often incomplete. We collected ticks from domestic or wild animals (generally a source of bushmeat) that were in contact with humans in 2 neighboring countries of tropical West Africa, Guinea and Liberia. In total, 382 ticks representing 6 species were collected in Liberia and 655 ticks representing 7 species were collected in Guinea. We found rickettsiae in 9 different species of ticks from both countries. *Rickettsia africae* was found in 93–100% of *Amblyomma variegatum*, in 14–93% of *Rhipicephalus* (*B.*) *geigyi*, *Rh.* (*B.*) *annulatus*, and *Rh.* (*B.*) *decoloratus*, and in several *Hyalomma marginatum rufipes* and *Haemaphysalis paraleachi*. A genetic variant of *R. africae* was found in *Amblyomma compressum*. *R. massiliae* was found in 10/61 (16%) of *Rh. senegalensis* ticks and in 2% of *Haemaphysalis paraleachi* ticks collected from dogs. We identified a new rickettsia in one of 44 (2%) *Ixodes muniensis* collected from a dog in Liberia. As this rickettsia is not yet isolated, we propose the provisional name "*Candidatus* Rickettsia liberiensis" (for the West African country where the host tick was collected).

Introduction

Tick-borne rickettsioses play an important role in public health (Raoult and Roux, 1997). The morbidity rate may be as high as 24.2 per 100,000 for Mediterranean spotted fever in endemic areas (Raoult et al., 1993). An incidence of rickettsial infection may be as high as 5.6% among travelers who developed an acute febrile infection after returning from sub-Saharan Africa. After malaria, it is the second most frequently identified etiology for systemic febrile illness among travelers (Jensenius et al., 2009; Freedman et al., 2006). A recent report showed that spotted-fever group rickettsioses may constitute up to 4.4% of acute non-malarial fevers in indigenous populations in Senegal (Socolovschi et al., 2010).

The causative agent of African tick-bite fever (ATBF) is *Rickettsia africae*, which is transmitted by *Amblyomma* sp. ticks (Kelly et al., 1996). These ticks are common in Western, Central and Southern Africa; they are aggressive and frequently bite humans (Walker et al., 2003; Nduaka and Ikeme, 1973). The *Amblyomma* sp. ticks were previously reported in Guinea (Balde, 1994; Kalivogi, 1986) and Cote d'Ivoire (Socolovschi et al., 2009), but not in Liberia. ATBF is probably the most common form of tick-transmitted rickettsiosis. *R. africae* has been isolated from *Amblyomma variegatum* in Cote d'Ivoire (Socolovschi et al., 2009)

Rickettsia raoultii (Mediannikov et al., 2008) is a pathogenic rickettsia that is epidemiologically associated with hard ticks of the genus *Dermacentor*. It was described in most European countries, Asiatic Russia, China, and North Africa (Mediannikov et al., 2008; Sarih et al., 2008), and it causes tick-borne lymphadenopathy (TIBOLA) in Europe (Parola et al., 2009).

Rickettsia massiliae is another rickettsia from the spotted fever group (SFG) that was repeatedly detected in *Rhipicephalus* sp. ticks in the Central African Republic, Mali, Algeria, Morocco, and Senegal (Mediannikov et al., 2010). The first human case of spotted fever caused by this rickettsia was identified in 2005 (Vitale et al., 2005).

The data on the distribution of rickettsial diseases in Africa are often fragmentary, with many countries and regions not studied at all. The sub-Saharan and Sahel regions of Africa have the highest seroprevalence for the rickettsiae that cause spotted fever (Tissot-Dupont et al., 1995). To our knowledge, no reports on ixodid ticks and tick-borne rickettsial diseases in Liberia have ever been published. In Guinea, several serological studies showed that the

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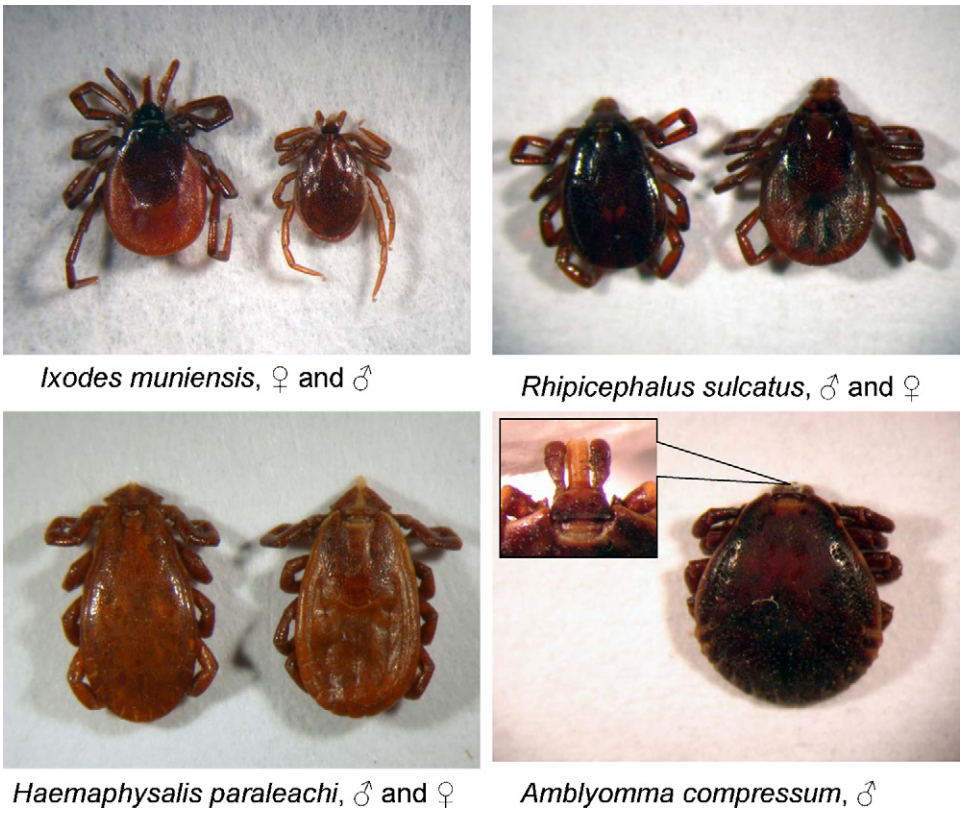

Fig. 1. Selected tick species collected and studied in Guinea and Liberia.

overall mean prevalence of antibodies against the SFG rickettsiae in the population is 10.5%, but may reach 25.5% (Faranah, Upper Guinea) (Kalivogi, 1986). In neighboring countries Sierra-Leone and Cote d'Ivoire, several studies were performed to determine the abundance of ticks and the presence of pathogenic rickettsiae in them (Berrelha et al., 2009; Redus et al., 1986).

Materials and methods

From March through April 2009, ticks were manually collected from domestic (cows, goats, and dogs) and wild (duiker, pangolin) animals in 4 villages of Liberia and 10 villages in Guinea. We sampled all animals available (all that were allowed to be sampled and present in the village at the moment of sampling). In total, 655 ticks were collected in Guinea and 382 in Liberia (Table 1). The ticks were then stored in 70% ethanol until identification and molecular study. Tick species and sex were identified according to standard taxonomic keys for adult ticks (Walker et al., 2003; Matthysse and Colbo, 1987). DNA from homogenized individual ticks was extracted using the BioRobot MDx Workstation (Qiagen, Courtaboeuf, France) with a customized extraction protocol according to the manufacturer's instructions and stored at 4 °C until use in PCR amplifications. Rickettsial DNA was initially detected by *Rickettsia* genus-specific qPCRs that were performed using LightCycler 2.0 equipment and software (Roche Diagnostics GmbH, Mannheim, Germany). Master mixtures were prepared according to the instructions of the manufacturer, and primers RKND03F and RKND03R and a probe, whose sequences are specific for the rickettsial citrate synthase (*gltA*) gene, were added to qPCRs as appropriate (Rolain et al., 2002).

All positive samples were subjected to *R. africae*-specific qPCR (Bechah et al., 2011). All samples that were positive by *Rickettsia* genus-specific, but negative by *R. africae*-specific qPCR were subjected to simple PCR as well as ticks positive for *R. africae*-specific qPCR (by 2 of each species from each location). The primers (Eurogentec, Seraing, Belgium) amplified an almost complete copy of the *gltA* gene, as previously described (Mediannikov et al., 2004; Roux et al., 1997). Additionally, a 632-bp fragment of the *ompA* gene was amplified using Rr. 190.70 and Rr. 190.701 primers (Fournier et al., 1998). The DNA extracted from uninfected ticks from colonies at the Unité des Rickettsies in Marseille, France, and sterile water were used as negative controls while the DNA extracted from the cell culture supernatant of *Rickettsia montanensis* served as a positive control. The PCR was performed in automated DNA thermal cyclers (GeneAmp 2400 and 9700; Applied Biosystems, Foster City, CA, USA). The amplicons were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and examined using an ultraviolet transilluminator. The PCR products were purified using a QIAquick Spin PCR Purification Kit (Qiagen) according to the manufacturer's instructions. The amplicons were sequenced using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems) with an ABI automated sequencer (Applied Biosystems).

The obtained sequences were assembled (ChromasPro 1.49 beta, Technelysium Pty Ltd., Tewantin, Australia) and compared with those available in GenBank by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the rickettsial genes were aligned and edited by BioEdit Sequence alignment editor v. 7.0.9.0 (Hall, 1999).

Results

In total, 382 ticks encompassing 6 species were collected from domestic and wild animals in Liberia, and 655 ticks representing 7 species were collected from domestic animals in Guinea (Table 1). All animals sampled were infested at least by one tick. *Haemaphysalis paraleachi* (Fig. 1) from dogs was the most abundant tick we collected in Liberia, and *Amblyomma variegatum* collected from cattle was the most abundant tick found in Guinea by our team.